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Genetic variation within the *ANGPTL4* gene is not associated with metabolic traits in white subjects at an increased risk for type 2 diabetes mellitus

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Abstract

Angiopoietin-like protein 4 (ANGPTL4) represents an adipokine with metabolic effects within adipose tissue, such as inhibition of lipoprotein lipase activity and stimulation of lipolysis. These effects were convincingly demonstrated in mice. Therefore, we asked whether genetic variation within the *ANGPTL4* gene contributes to prediabetic phenotypes, such as dyslipidemia, insulin resistance, or β-cell dysfunction, in white subjects at an increased risk for type 2 diabetes mellitus. We genotyped 629 subjects with and without a family history of diabetes for the 4 single nucleotide polymorphisms (SNPs) rs4076317, rs2278236, rs1044250, and rs11672433 and performed correlational analyses with metabolic traits. For metabolic characterization, all subjects underwent an oral glucose tolerance test; a subset was additionally characterized by hyperinsulinemic-euglycemic clamp. The 4 SNPs rs4076317, rs2278236, rs1044250, and rs11672433 cover 100% of common genetic variation (minor allele frequency ≥0.05) within the *ANGPTL4* gene ($r^2 \geq 0.8$). None of these SNPs revealed significant correlation with anthropometric data (sex, age, body mass index, body fat, and waist-hip ratio) or with family history of diabetes. Furthermore, no reliable correlations were found with fasting triglycerides, fasting nonesterified fatty acids, and area under the curve of nonesterified fatty acids during oral glucose tolerance test or with parameters of insulin sensitivity and insulin secretion. Finally, haplotype analysis revealed the existence of 8 common diplotypes. None of these, however, was significantly correlated with insulin sensitivity, insulin secretion, or plasma lipid measures. We conclude that common genetic variation within the *ANGPTL4* gene may not play a major role in the development of prediabetic phenotypes in our white population.

1. Introduction

About 6 years ago, angiopoietin-like protein 4 (ANGPTL4) was independently described by 3 groups and originally named *hepatic fibrinogen/angiopoietin-related protein* [1], *fasting-induced adipose factor* [2], and *PPARγ target gene related to angiopoietin* [3]. Angiopoietin-like protein 4 was characterized as an adipokine, a metabolically relevant factor predominantly secreted by white adipose tissue. However, substantial expression levels were additionally detected in skeletal muscle, liver, heart, and intestine [1-3].

Structurally, ANGPTL4 belongs to the angiopoietin/ANGPTL family. This protein family up to now comprises at least 10 members with a molecular weight of 44 to 58 kd that are characterized by an N-terminal isoform-specific coiled-coil domain and a conserved C-terminal fibrinogen-like motif. The latter allows oligomerization. The 3 angiopoietins (angiopoietin 1, 2, and 4) were shown to modulate angiogenesis via binding to the endothelial Tie2 receptor tyrosine kinase. By contrast, the ANGPTL proteins are not involved in the regulation of angiogenesis. Furthermore, the receptors for ANGPTL proteins as well as the signaling pathways initiated by ANGPTL proteins in target cells are still unknown. Among the 7 ANGPTL

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proteins described to date, ANGPTL3, ANGPTL4, and ANGPTL6 appear to be of metabolic relevance by modulating adipose tissue functions and body fat mass (reviewed by Kersten [4]).

The molecular function of the adipokine ANGPTL4 was extensively, but exclusively, studied in mice by injection [5], targeted gene knockout [6], as well as transgenic [6,7] and adenoviral overexpression [8,9]. Within adipose tissue, ANGPTL4 obviously exerts auto-/paracrine actions and affects lipid metabolism: (*a*) via inhibition of lipoprotein lipase activity [6], ANGPTL4 blocks the clearance of very low-density lipoproteins and chylomicrons and thus provokes hypertriglyceridemia [5-9]; (*b*) ANGPTL4 stimulates adipose tissue lipolysis by induction of adipose triglyceride lipase/desnutrin expression [10], resulting in elevated plasma glycerol and nonesterified fatty acid (NEFA) levels [5,10]. Besides hyperlipidemia, ANGPTL4 promotes adipose tissue weight loss and hepatic steatosis [9,10]. Importantly, ANGPTL4 was consistently found up-regulated in mouse models of obesity and type 2 diabetes mellitus [3].

Very recently, the first genetic study that assessed the association of nonsynonymous mutations within the coding region of the *ANGPTL4* gene with metabolic traits in 3 study populations was published [11]. The authors identified a very rare mutation (minor allele frequency [MAF] = 0.013), E40K, in European Americans that was systematically associated with lower plasma triglyceride levels in all 3 populations. This clearly provides evidence that ANGPTL4 plays an important role in human lipid metabolism. Dyslipidemia, insulin resistance, and β-cell dysfunction are early steps in the pathogenesis of the polygenic disease type 2 diabetes mellitus. Because ANGPTL4 appears to play a role in human lipid metabolism, we assessed the importance of its gene as a prediabetes candidate gene. We focused on common genetic variations (MAF $\geq$0.05) because rare mutations, such as E40K, are not expected to essentially contribute to the epidemic of type 2 diabetes mellitus and studied their impact on the above-mentioned prediabetes phenotypes.

2. Patients and methods

2.1. Subjects

The 629 nondiabetic subjects (358 women, 271 men) with and without a family history of diabetes mellitus were recruited from the southern part of Germany and participated in the ongoing Tübingen Family Study for type 2 diabetes mellitus and the Tuebingen Lifestyle Intervention Program described in Stefan et al [12]. All subjects were metabolically characterized by oral glucose tolerance test (OGTT); a subgroup of 201 subjects was additionally characterized by hyperinsulinemic-euglycemic clamp. The participants gave informed written consent to the study. The protocol was approved by the local ethical committee.

2.2. Analysis of the ANGPTL4 gene, selection of single nucleotide polymorphisms for genotyping, and generation of diplotypes (haplotype pairs)

Using the publicly available phase II data of the International HapMap Project derived from a population of Utah residents with ancestry from northern and western Europe (release 21, July 2006, http://www.hapmap.org/index.html.en [13]), we screened in silico the complete *ANGPTL4* gene spanning 10.25 kilobases (kb) (8 exons, 7 introns, located on human chromosome 19p13.3) as well as 5 kb and 2.5 kb of its 5′- and 3′-flanking regions, respectively (Fig. 1). Based on HapMap genotype and linkage disequilibrium (LD) data, a single 9-kb LD block spanning the whole region was defined by the "solid spine of LD" mode of the Haploview software (http://www.broad.mit.edu/mpg/haploview [14]). Within this LD block, 6 informative single nucleotide polymorphisms (SNPs) were present (Fig. 1); and the D' values of all the SNP pairs were 1.0 (r^2 values given in Fig. 1). One SNP, that is, rs10404615, was rare (MAF = 0.019) and therefore was not genotyped. Among the 5 common SNPs (MAF >0.16, all), SNP rs2278236 was in complete linkage with rs7255436 (D' = 1.0, r^2 = 1.0) and therefore was chosen as representative for both. As marked in Fig. 1, the 4 genotyped SNPs were rs4076317 G/C (12 nucleotides upstream of the transcription initiation site), rs2278236 T/C (located within intron 3), rs1044250 C/T (located within exon 6 and translated into a Thr266Met amino acid exchange), and rs11672433 G/A (located within exon 7, reflecting a silent Pro389Pro mutation). These SNPs covered 100% of common variants (MAF $\geq$0.05) within the *ANGPTL4* gene, with an $r^2 \geq 0.8$ according to Tagger analysis (http://www.broad.mit.edu/mpg/tagger). Inferred diplotypes (haplotype pairs) derived from these SNPs were generated by the HPlus software (http://qge.fhcrc.org/hplus [15]).

2.3. Genotyping of the study population

For genotyping, DNA was isolated from whole blood using a commercial DNA isolation kit (NucleoSpin; Macherey & Nagel, Düren, Germany). The SNPs were genotyped using the TaqMan assay (Applied Biosystems, Foster City, CA). The TaqMan genotyping reaction was amplified on a GeneAmp PCR system 7000 (Applied Biosystems) (50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute), and fluorescence was detected on an ABI Prism sequence detector (Applied Biosystems). Quality control was performed in the manner reported recently [12]. For SNP rs2278236, the TaqMan method failed. This SNP was therefore genotyped by direct sequencing of the corresponding polymerase chain reaction product. The overall genotyping success rate was 97.3% (rs4076317, 96.5%; rs2278236, 100.0%; rs1044250, 96.5%; and rs11672433, 96.2%), and rescreening of 3% of subjects gave 100% identical results.

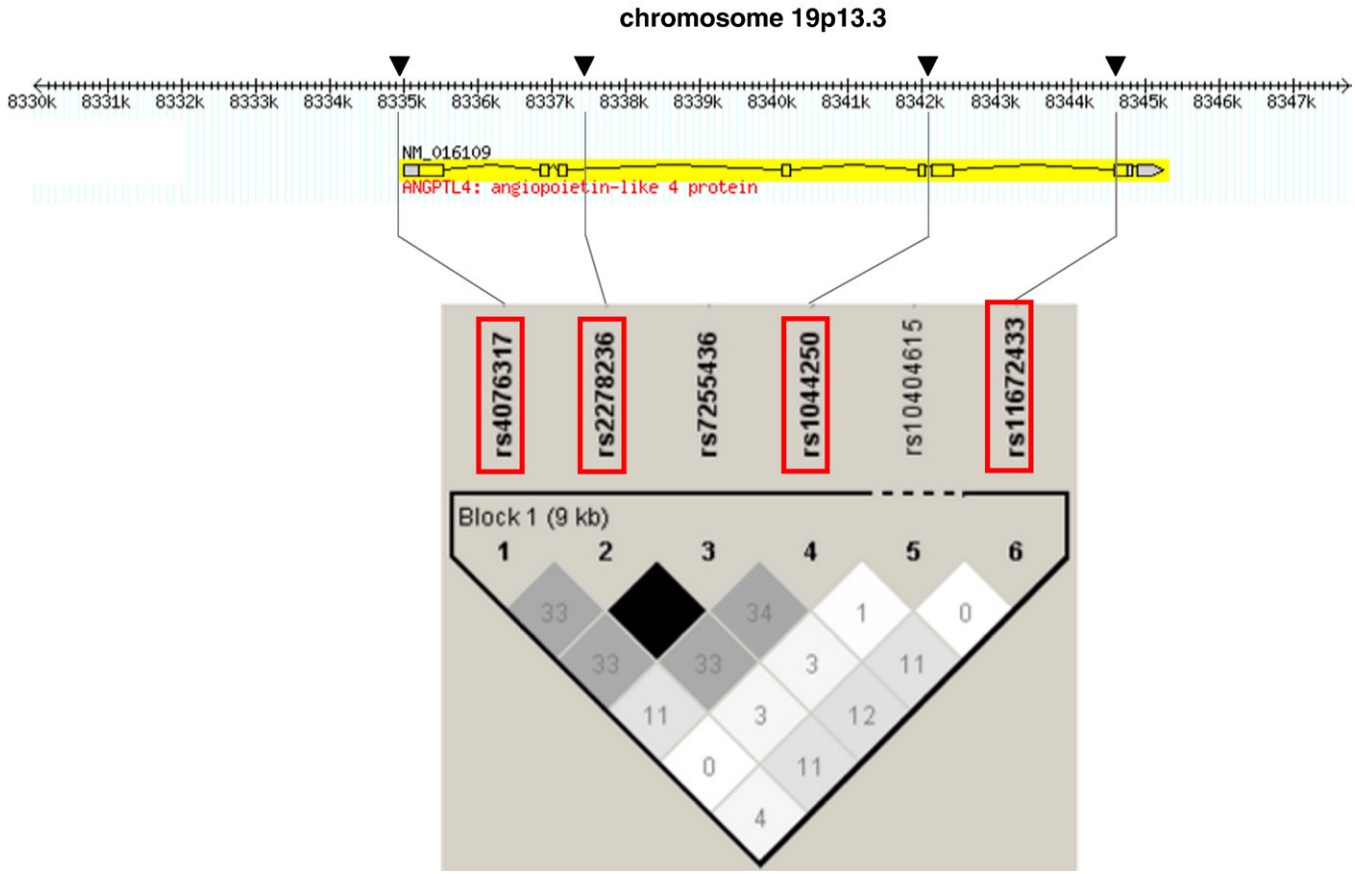

Fig. 1. Genomic region of human chromosome 19 harboring the *ANGPTL4* gene and LD data of informative SNPs within this region (HapMap data). The *ANGPTL4* gene consists of 8 exons and 7 introns and spans 10.25 kb from nucleotide 8 335 010 to nucleotide 8 345 256. The locations of the genotyped SNPs rs4076317, rs2278236, rs1044250, and rs11672433 are indicated by arrows. The 9-kb LD block was defined by the solid spine of LD mode of the Haploview software. The Haploview LD color scheme "*r*-squared" was chosen to visualize regions of high linkage (black diamond: $r^2 = 1.0$). Within the diamonds, the r^2 values are given.

2.4. Body composition and body fat distribution

Percentage of body fat, body mass index (BMI), and waist and hip circumferences were measured as described earlier [12].

2.5. OGTT and hyperinsulinemic-euglycemic clamp

Both assays were performed as formerly described in detail [12].

2.6. Determination of blood parameters

Plasma glucose, insulin, C-peptide, and NEFA concentrations were measured as recently described [12]. Plasma triglycerides were measured with a standard colorimetric method using a Roche/Hitachi analyzer (Roche Diagnostics, Mannheim, Germany).

2.7. Calculations

The area under the curve (AUC) of plasma glucose levels during the OGTT was calculated as $0.5h \cdot (0.5 \cdot Glc_0 + Glc_{30} + Glc_{60} + Glc_{90} + 0.5 \cdot Glc_{120})$. The AUC of plasma insulin and NEFA levels during OGTT were calculated analogously. Homeostasis model assessment of insulin resistance (HOMA-IR, in arbitrary units) was calculated as $(2 \cdot Glc_0 \cdot Ins_0)/45$. First-phase insulin secretion (in picomoles per liter), insulin sensitivity from the OGTT (in arbitrary units), and clamp-derived insulin sensitivity (in arbitrary units) were calculated as reported earlier [12].

2.8. Statistical analyses

Usually, data are given as means ± SE. Log transformation of metabolic variables was performed before simple and multivariate linear regression analyses. In multivariate linear regression models, the trait was chosen as dependent variable; and sex, age, body fat, and genotype were tested as independent variables. A P value < .05 was considered statistically significant. The statistical software package JMP 4.0 (SAS, Cary, NC) was used. Statistical retrospective power was calculated from log-transformed and adjusted data using the power calculation mode of JMP 4.0's multivariate linear regression model with the trait's standard deviation (σ) and an effect size (δ) of $\sigma/5$. Single nucleotide polymorphism LD analysis (D', r^2) was performed using the JLIN program provided by the Western Australian Institute

for Medical Research (http://www.genepi.org.au/jlin [16]). Hardy-Weinberg equilibrium was tested using χ^2 test.

3. Results

3.1. Characterization and genotyping of a white population at an increased risk for type 2 diabetes mellitus

We genotyped 629 overweight nondiabetic subjects from the southwest of Germany whose clinical characteristics are presented in Table 1; 76.8% of the subjects had a family history of diabetes, that is, at least one second-degree relative with type 2 diabetes mellitus. The MAF data are presented in Table 2; the genotype distribution is given in Tables 3 and 4. Differences between the observed MAF and the MAF published by HapMap might be due to genuine differences between our German population and HapMap's cohort of Utah residents with northern and western European ancestry or our cohort's greater sample size (N = 629 vs HapMap N = 120). All 4 SNPs were found to be in Hardy-Weinberg equilibrium (rs4076317, P = .5; rs2278236, P = .5; rs1044250, P = .3; and rs11672433, P = .22), and the LD statistics are given in Table 2.

3.2. Correlations of ANGPTL4 SNPs with anthropometric data, family history of diabetes, and metabolic traits

The SNPs rs4076317, rs2278236, rs1044250, and rs11672433 did neither correlate with anthropometric data, such as sex, age, BMI, body fat content, and waist-hip ratio, nor with family history of diabetes (data not shown). All 629 subjects were metabolically characterized by OGTT; 201 out of them were additionally characterized by hyperinsulinemic-euglycemic clamp. As presented in Tables 3 and 4, SNPs rs4076317, rs2278236, and rs11672433 were not significantly correlated with parameters of insulin sensitivity or insulin secretion. Correlations of borderline significance were detected between SNP rs1044250 and the clamp-derived insulin sensitivity index (ISI) in the additive model (P = .0345, analysis of variance; Table 4) and the OGTT-derived ISI in the dominant model (P = .0291, analysis of variance; Table 4). However, no additivity in the minor allele's effect was observed. Furthermore, the levels of significance would not withstand correction for multiple comparisons. The SNPs rs2278236, rs1044250, and rs11672433 were not correlated with fasting plasma triglycerides, fasting plasma NEFA, or the AUC of plasma NEFA levels during OGTT (Tables 3 and 4). The SNP rs4076317 revealed significant correlations with fasting plasma NEFA and the AUC of plasma NEFA levels during OGTT in the dominant model only (Table 3). Again, no additivity in the minor allele's effect on fasting plasma NEFA levels was observed; and the correlations would not withstand correction for multiple comparison.

Table 1
Clinical characteristics of the study population (N = 629)

	Women	Men
n	358	271
Family history of diabetes (%)	80.2	72.3
Age (y)	40.5 ± 0.6	39.9 ± 0.8
BMI (kg/m^2)	27.5 ± 0.3	27.2 ± 0.3
Body fat content (%)	33.2 ± 0.5	22.5 ± 0.5
Waist-hip ratio	0.824 ± 0.004	0.922 ± 0.005

Data are given as means (±SE).

Table 2
Linkage disequilibrium statistics (D', r^2) among the 4 genotyped SNPs of the 17.75-kb genomic region harboring the *ANGPTL4* gene

D' values (above empty cells), r^2 values (below empty cells)				
SNP	rs4076317	rs2278236	rs1044250	rs11672433
rs4076317	–	0.945	0.985	1.000
rs2278236	0.429	–	0.993	0.951
rs1044250	0.178	0.377	–	1.000
rs11672433	0.086	0.161	0.077	–
$MAF_{observed}$	0.311	0.485	0.289	0.159
MAF_{HapMap}	0.161	0.375	0.358	0.172

3.3. Correlations of ANGPTL4 SNP diplotypes with metabolic traits

Because all analyses so far did not point to a major contribution of genetic variation within the *ANGPTL4* gene to metabolic traits, we generated inferred diplotypes (haplotype pairs) from the 4 genotyped SNPs using the HPlus software; and the 8 most common diplotypes (frequency >0.05) were chosen for correlational analyses. No significant correlations were found between any of the common diplotypes and plasma lipid parameters, indices of insulin sensitivity, or parameters of insulin secretion (data not shown).

4. Discussion

Using publicly available HapMap data, we identified a single 9-kb high-LD block that comprehensively covers the whole *ANGPTL4* locus. Genotyping of a metabolically well-characterized population for the 4 common SNPs—rs4076317 G/C, rs2278236 T/C, rs1044250 C/T (Thr266Met), and rs11672433 G/A—covering 100% of common genetic variation (MAF ≥0.05) within this high-LD block with an $r^2 \geq 0.8$ revealed no reliable association with anthropometric data, family history of diabetes, plasma triglyceride and NEFA levels, insulin sensitivity, or insulin secretion when analyzed separately or in the form of haplotype pairs (diplotypes). A limitation of our study is that we only analyzed effects sizes ≥0.2 standard deviations (after adjustment of the trait for known confounding variables) with sufficient power (≥80 %). Thus, smaller effects of *ANGPTL4* SNPs remained undetected in this study. However, we assume that SNPs with effect sizes <0.2 standard deviations do not play a major role in the development of prediabetic phenotypes. Nevertheless, further replications in other cohorts are needed to confirm

Table 3
Correlations of SNPs rs4076317 G/C and rs2278236 T/C with metabolic parameters (N = 629)

SNP genotype	rs4076317			$P_{additive}$ (power)	$P_{dominant}$ (power)	rs2278236			$P_{additive}$ (power)	$P_{dominant}$ (power)
	GG	GC	CC			TT	TC	CC		
n	293	279	56	–	–	162	324	143	–	–
Glucose, fasting (mmol/L)	5.03 ± 0.04	5.14 ± 0.04	5.34 ± 0.10	.12 (0.995)	.14 (0.998)	5.09 ± 0.06	5.06 ± 0.04	5.22 ± 0.06	.22 (0.995)	.5 (0.998)
Glucose, AUC OGTT (mmol/L)	14.8 ± 0.2	14.9 ± 0.2	15.4 ± 0.5	.8 (0.995)	.6 (0.998)	15.0 ± 0.3	14.8 ± 0.2	14.8 ± 0.3	.8 (0.995)	.9 (0.998)
C-peptide, 30-min OGTT (pmol/L)	1926 ± 47	1953 ± 48	1950 ± 108	.8 (0.995)	.7 (0.998)	1960 ± 63	1952 ± 45	1887 ± 68	.7 (0.995)	.8 (0.998)
1st-phase insulin secretion (pmol/L)	1114 ± 38	1111 ± 39	1095 ± 88	.4 (0.995)	.18 (0.998)	1138 ± 51	1110 ± 36	1080 ± 55	1.0 (0.995)	.8 (0.998)
Insulin, fasting (pmol/L)	53.3 ± 2.2	55.2 ± 2.2	57.5 ± 4.9	.5 (0.995)	.4 (0.998)	55.7 ± 2.9	53.7 ± 2.1	54.8 ± 3.1	.9 (0.995)	.7 (0.998)
HOMA-IR (U)	2.02 ± 0.10	2.19 ± 0.10	2.34 ± 0.22	.3 (0.995)	.3 (0.998)	2.15 ± 0.13	2.05 ± 0.09	2.26 ± 0.14	.8 (0.995)	.8 (0.998)
ISI, OGTT (U)	17.7 ± 0.6	17.8 ± 0.7	16.5 ± 1.5	.7 (0.995)	1.0 (0.998)	17.3 ± 0.9	17.5 ± 0.6	18.2 ± 0.9	.8 (0.995)	.7 (0.998)
ISI, clamp (U)[a]	0.109 ± 0.006	0.112 ± 0.007	0.124 ± 0.017	.5 (0.713)	.5 (0.806)	0.120 ± 0.009	0.108 ± 0.006	0.108 ± 0.009	.22 (0.713)	.12 (0.806)
NEFA, fasting (μmol/L)	583 ± 14	547 ± 14	574 ± 32	.11 (0.995)	.0341 (0.998)	574 ± 18	567 ± 13	555 ± 20	.7 (0.995)	.4 (0.998)
NEFA, AUC OGTT (h·μmol/L)	459 ± 12	439 ± 12	414 ± 27	.06 (0.994)	.0253 (0.998)	453 ± 16	453 ± 11	425 ± 17	.5 (0.994)	.6 (0.998)
Triglycerides, fasting (mg/dL)	109 ± 9	141 ± 10	97 ± 22	.07 (0.995)	.3 (0.998)	110 ± 13	127 ± 9	126 ± 14	.21 (0.995)	.5 (0.998)

Raw data are presented and given as means ± SE. For statistical analysis, data were log transformed; C-peptide (30-minute OGTT) and first-phase insulin secretion were adjusted for sex, age, body fat, and insulin sensitivity (OGTT); all other data were adjusted for sex, age, and body fat. Below the *P* values, the statistical power based on the trait's standard deviation (σ) and an effects size (δ) of $\sigma/5$ is given in brackets.

[a] n = 201.

Table 4
Correlations of SNPs rs1044250 C/T (Thr266Met) and rs11672433 G/A with metabolic parameters (N = 629)

SNP genotype	rs1044250			$P_{additive}$ (power)	$P_{dominant}$ (power)	rs11672433			$P_{additive}$ (power)	$P_{dominant}$ (power)
	CC	CT	TT			GG	GA	AA		
n	322	249	57	–	–	447	159	20	–	–
Glucose, fasting (mmol/L)	5.15 ± 0.04	5.05 ± 0.05	5.07 ± 0.10	.17 (0.995)	.06 (0.998)	5.12 ± 0.03	5.08 ± 0.06	5.15 ± 0.16	.8 (0.995)	.5 (0.998)
Glucose, AUC OGTT (mmol/L)	15.1 ± 0.2	14.7 ± 0.3	14.6 ± 0.5	.25 (0.995)	.10 (0.998)	14.9 ± 0.2	14.8 ± 0.3	16.1 ± 0.9	.4 (0.995)	.9 (0.998)
C-peptide, 30-min OGTT (pmol/L)	1948 ± 45	1948 ± 51	1861 ± 106	.4 (0.995)	.3 (0.998)	1932 ± 38	1957 ± 64	2008 ± 177	.3 (0.995)	.4 (0.998)
1st-phase insulin secretion (pmol/L)	1129 ± 37	1085 ± 42	1123 ± 87	.8 (0.995)	.9 (0.998)	1108 ± 31	1108 ± 52	1206 ± 147	.7 (0.995)	.9 (0.998)
Insulin, fasting (pmol/L)	56.9 ± 2.1	51.6 ± 2.3	54.0 ± 4.9	.24 (0.995)	.12 (0.998)	54.8 ± 1.7	53.8 ± 2.9	57.3 ± 8.2	.8 (0.995)	.6 (0.998)
HOMA-IR (U)	2.26 ± 0.09	1.96 ± 0.11	2.09 ± 0.22	.15 (0.995)	.07 (0.998)	2.15 ± 0.08	2.06 ± 0.13	2.23 ± 0.37	.8 (0.995)	.5 (0.998)
ISI, OGTT (U)	17.4 ± 0.6	18.0 ± 0.7	17.4 ± 1.5	.09 (0.995)	.0291 (0.998)	17.5 ± 0.5	17.9 ± 0.9	16.7 ± 2.5	.9 (0.995)	.7 (0.998)
ISI, clamp (U) [a]	0.111 ± 0.006	0.106 ± 0.007	0.134 ± 0.014	.0345 (0.713)	.3 (0.806)	0.110 ± 0.005	0.113 ± 0.009	0.130 ± 0.037	.8 (0.713)	.6 (0.806)
NEFA, fasting (μmol/L)	558 ± 13	566 ± 15	615 ± 31	.09 (0.995)	.06 (0.998)	569 ± 11	555 ± 19	607 ± 51	.4 (0.995)	.4 (0.998)
NEFA, AUC OGTT (h·μmol/L)	449 ± 11	439 ± 13	466 ± 26	.8 (0.994)	.8 (0.998)	440 ± 9	455 ± 16	510 ± 43	.6 (0.994)	.5 (0.998)
Triglycerides, fasting (mg/dL)	124 ± 9	124 ± 10	106 ± 21	.5 (0.995)	.9 (0.998)	125 ± 8	117 ± 13	105 ± 35	.5 (0.995)	.5 (0.998)

Raw data are presented and given as means ± SE. For statistical analysis, data were log transformed; C-peptide (30-minute OGTT) and first-phase insulin secretion were adjusted for sex, age, body fat, and insulin sensitivity (OGTT); all other data were adjusted for sex, age, and body fat. Below the P values, the statistical power based on the trait's standard deviation (σ) and an effects size (δ) of $\sigma/5$ is given in brackets.

[a] n = 201.

the lack of major influences of genetic variation within this locus on metabolic traits in the white population.

In conclusion, our data suggest that common genetic variation within the *ANGPTL4* gene may not play a major role in the development of prediabetic phenotypes, such as dyslipidemia, insulin resistance, or β-cell dysfunction, in our white population at an increased risk for type 2 diabetes mellitus.

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References

[1] Kim I, Kim HG, Kim H, et al. Hepatic expression, synthesis and secretion of a novel fibrinogen/angiopoietin-related protein that prevents endothelial-cell apoptosis. Biochem J 2000;346:603-10.

[2] Kersten S, Mandard S, Tan NS, et al. Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator–activated receptor target gene. J Biol Chem 2000;275:28488-93.

[3] Yoon JC, Chickering TW, Rosen ED, et al. Peroxisome proliferator–activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation. Mol Cell Biol 2000;20:5343-9.

[4] Kersten S. Regulation of lipid metabolism via angiopoietin-like proteins. Biochem Soc Trans 2005;33:1059-62.

[5] Yoshida K, Shimizugawa T, Ono M, et al. Angiopoietin-like protein 4 is a potent hyperlipidemia-inducing factor in mice and inhibitor of lipoprotein lipase. J Lipid Res 2002;43:1770-2.

[6] Koster A, Chao YB, Mosior M, et al. Transgenic angiopoietin-like (angptl)4 overexpression and targeted disruption of angptl4 and angptl3: regulation of triglyceride metabolism. Endocrinology 2005; 146:4943-50.

[7] Yu X, Burgess SC, Ge H, et al. Inhibition of cardiac lipoprotein utilization by transgenic overexpression of Angptl4 in the heart. Proc Natl Acad Sci U S A 2005;102:1767-72.

[8] Ge H, Yang G, Yu X, et al. Oligomerization state-dependent hyperlipidemic effect of angiopoietin-like protein 4. J Lipid Res 2004;45:2071-9.

[9] Xu A, Lam MC, Chan KW, et al. Angiopoietin-like protein 4 decreases blood glucose and improves glucose tolerance but induces hyperlipidemia and hepatic steatosis in mice. Proc Natl Acad Sci U S A 2005; 102:6086-91.

[10] Mandard S, Zandbergen F, van Straten E, et al. The fasting-induced adipose factor/angiopoietin-like protein 4 is physically associated with lipoproteins and governs plasma lipid levels and adiposity. J Biol Chem 2006;281:934-44.

[11] Romeo S, Pennacchio LA, Fu Y, et al. Population-based resequencing of ANGPTL4 uncovers variations that reduce triglycerides and increase HDL. Nat Genet 2007;39:513-6.

[12] Stefan N, Machicao F, Staiger H, et al. Polymorphisms in the gene encoding adiponectin receptor 1 are associated with insulin resistance and high liver fat. Diabetologia 2005;48:2282-91.

[13] The International HapMap Project. Nature 2003;426:789-96.

[14] Barrett JC, Fry B, Maller J, et al. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263-5.

[15] Li SS, Khalid N, Carlson C, et al. Estimating haplotype frequencies and standard errors for multiple single nucleotide polymorphisms. Biostatistics 2003;4:513-22.

[16] Carter KW, McCaskie PA, Palmer LJ. JLIN: a java based linkage disequilibrium plotter. BMC Bioinformatics 2006;7:60.